THE PASSAGE OF HAEMOGLOBIN THROUGH THE KIDNEYS

BY

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If an isotonic solution of haemoglobin obtained from rabbit's red blood cells be injected into a vein of a normal rabbit, the urine is found to be tinged with haemoglobin a few minutes after the injection. Whilst performing experiments devised with a view to studying the mechanism of production of suppression of urine in blackwater fever, the opportunity was afforded of examining a large number of kidneys removed from the animals at various periods after intravenous injection of haemoglobin solutions.

It appeared possible that a careful study of this material might throw some light upon the manner in which haemoglobin passes through the kidneys from the blood stream to the urine.

Attention has already been drawn by Yorke and Nauss (1911) in a previous communication to the fact that under certain conditions suppression of urine follows the intravenous injection of large amounts of homologous haemoglobin. Microscopical examination of the kidneys in such cases shows the condition to be due to occlusion of the renal tubules by casts. These plugs are of different appearance, according as the kidney examined is obtained from animals which have succumbed shortly after the injection of haemoglobin or several days later. When seen in kidneys obtained within a few hours of the injection they are but slightly granular and appear to consist of a fairly homogeneous material, which, when unstained, is of a brownish colour. When stained with various dyes they react in much the same manner as do the red blood cells, e.g., with eosin they stain a brownish pink and with van Gieson a brownish red, whilst with Heidenhain's iron alum haematoxylin method they are dyed a dark blue-black colour.

The casts found in those kidneys where death occurred several days after the intravenous injection of haemoglobin are, as a rule, exceedingly granular. The granules vary considerably in size, from being mere points to granules of about 2 μ in diameter. These granular casts possess in the main the same staining characteristics as the previous variety. Sometimes in the later stages certain of the casts were found to have become in part crystalline.

As to the precise nature of these plugs one is unable to speak with certainty. They are only found after intravenous injection of haemoglobin, and from their appearance both unstained and also after staining with various dyes there appears to be no doubt but that they are derived from haemoglobin. Either they consist of haemoglobin itself supported by a mucoid basis, or they are composed of some closely related derivative. I was able to obtain the iron reaction neither with potassium ferrocyanide and hydrochloric acid (Berlin blue reaction) nor by first treating with ammonium sulphide and then subsequently with hydrochloric acid and potassium ferrocyanide (Turnbull's blue reaction). Possibly the failure to obtain this reaction was because the process of disintegration had not continued sufficiently far for the setting free of iron from albuminous combination to have occurred. It is interesting, however, to note in this connection that the iron reaction was obtained by Werner (1907) in the case of the casts found in the kidneys of several human beings who had succumbed from suppression of urine following blackwater fever. Here, however, the individuals had survived the attacks of haemoglobinuria by over a week. A careful examination of the exact site in which the casts occurred revealed the fact that they were limited to the renal tubules, and were never to be found in the meniscus of Bowman's capsules.

Plugs were frequently found in all portions of the tubule, with the single exception of the glomeruli. The fact that the kidneys removed from over thirty animals, at periods varying from one hour to over eight days after the injection of widely different amounts of haemoglobin, were examined, without discovering casts in the glomeruli in a single instance, is highly suggestive that haemoglobin does not escape through Bowman's capsules.

The possibility suggests itself that haemoglobin might in reality

be excreted by means of Bowman's capsules, but that it is so quickly carried away by the rapid stream of water escaping from the tufts that it is not recognised. However, even assuming Ludwig's theory, that the constituents of the urine are filtered through the capsular tufts in the proportion in which they exist in the blood plasma, to be correct, then in certain of our experiments the glomerular filtrate must have contained at least 8 to 12 per cent. of haemoglobin. If such a solution of haemoglobin be spread in a fairly thick film—considerably less, however, than the thickness of the section of the kidney employed—on a glass slide, fixed immediately before drying has occurred, and then stained with Heidenhain's iron alum haematoxylin or van Gieson, a highly-coloured granular appearance is produced, somewhat resembling the casts seen in the tubules. Such appearances were never observed in the meniscus of the Bowman's capsule in our experimental animals. Moreover, even in those cases in which suppression of urine had occurred and where presumably it would be impossible for any haemoglobin which escaped through Bowman's capsules (sometimes in these cases considerably dilated) to have been washed away, no casts were found in the meniscus of the capsules.

Examination of the kidneys of three blackwater fever patients who had succumbed from suppression of urine revealed an exactly comparable state of affairs. Casts being constantly found in all portions of the renal tubules, except in the meniscus of Bowman's capsules. The same was observed in the kidneys of dogs which died during the passage of haemoglobin resulting from infection with *Piroplasma canis*.

It is difficult to determine the exact portion of the renal tubule which is responsible for the excretion of haemoglobin Presumably, however, it is the epithelium of the convoluted tubules and possibly also that of the tubes of Henle, as in sections of kidneys removed within a few hours of the intravenous injection of haemoglobin the casts are found to be limited to the cortex, and are not seen in the large collecting tubes of Bellini. Later, however, the plugs are found in the large collecting tubules, but in these cases they have probably simply descended from higher portions of the tubules.

Again, it is interesting in this connection to compare the percentage of haemoglobinuria in relation to the percentage of

haemoglobinaemia observed at definite intervals after intravenous injection of haemoglobin. In Figures 1 and 2 the results of two such observations are given. It is at once apparent from a study of these graphs that the curve representing the percentage of haemoglobin passed in the urine does not run at all parallel with that representing the degree of haemoglobinaemia, for, whereas the latter falls in a comparatively regular manner from the time of injection until the end of the experiment, the former does not reach its maximum until after the lapse of some hours (4 to 5) and, moreover, the curve is irregular, neither attaining its maximum nor falling to zero by regular gradations.

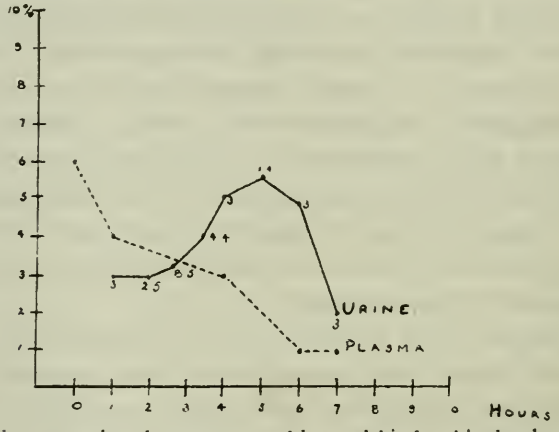


FIG. 1.—Graph representing the percentage of haemoglobin found in the plasma and urine after intravenous injection of an isotonic solution of homologous haemoglobin. The figures along the curve representing the degree of haemoglobinuria indicate the amount of urine passed.

Although the degree of haemoglobinuria varies to a certain extent inversely as the volume of urine passed, nevertheless, this factor alone is insufficient to explain the phenomenon that the maximum amount of haemoglobinuria is not attained for several hours after the intravenous injection of haemoglobin. Assuming that haemoglobin is filtered through the glomeruli, according to Ludwig's view, then one would expect that the percentage found in the urine would depend upon the following two factors. Firstly, it would vary directly with the degree of haemoglobinaemia, and, secondly, it would vary indirectly with the volume of urine passed into the bladder, or, in other words, with the amount of concentration that occurred during the passage of the urine through the convoluted tubules. This, however, does not appear to be the case. Furthermore, it was observed that when several large amounts of

haemoglobin were injected into the same animal at stated intervals, the degree of haemoglobinuria resulting from the last injection was usually much lower than that following the first injection, even though the degree of haemoglobinaemia in this case was not so great as that resulting from the last injection. Moreover, as in these cases the volume of urine excreted after several injections was greatly diminished in amount, owing to partial suppression having occurred, the lower percentage of haemoglobinuria observed could not result from the secretion of a large quantity of watery urine dependent upon decreased absorption of water as it passed through the uriniferous tubules.

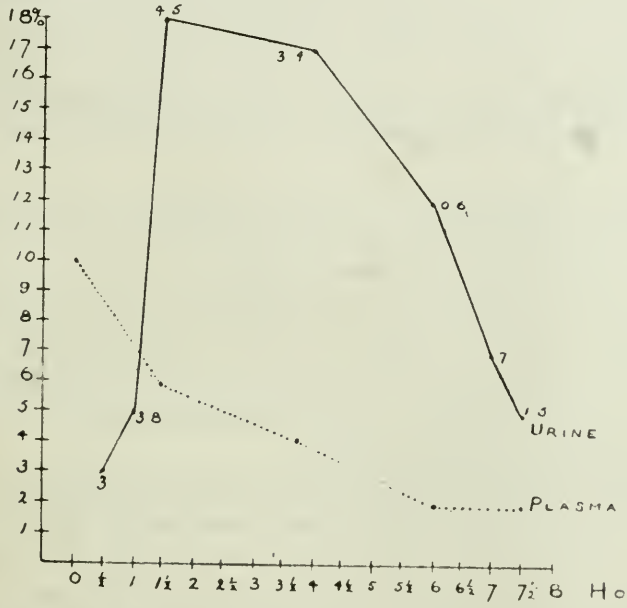


FIG. 2.—Graph representing the percentage of haemoglobin present in the plasma and urine after intravenous injection of an isotonic solution of homologous haemoglobin. The numbers along the curve representing the degree of haemoglobinuria indicate the amount of urine passed.

It would seem that these observations are more in harmony with the view that haemoglobin is secreted by the renal epithelium than that it is filtered through the glomeruli, and, that the amount of haemoglobin eliminated into the urine is dependent upon the activity of the epithelium lining the renal tubules.

There is, moreover, additional evidence which affords support to this view, and that is obtained by examination of the epithelium lining the capsules and various portions of the uriniferous tubules.

On examining sections of the kidneys of pups, which have died from *Piroplasma canis* during the passage of haemoglobin, fixed in formalin and stained with Heidenhain's iron alum haematoxylin method, one is at once struck by the presence of large numbers of darkly staining granules in the epithelium of the renal tubules. These granules vary in size, some being exceedingly small and others much larger, some of the latter being 2 to 3μ in diameter. In sections stained with Delafield's haematoxylin and van Gieson's solution, the granules are of a pale brownish pink colour. These intracellular granules resemble very closely in appearance the granules composing the casts in the lumen of the tubules. They were only found in the cortical region of the kidney, and appeared to be almost entirely limited to the epithelium lining the convoluted tubules. They were never observed in the flattened epithelium of the glomeruli nor in the epithelium lining the collecting tubules of Bellini.

Analogous appearances were observed in the sections of the kidneys of the experimental rabbits, but here the granules were not present in such large numbers as in the dogs.

As in the case of the plugs found in the lumen of the tubules, one is at present unable to decide as to the exact nature of these granules. There appears, however, to be but little doubt that they are derived, in part, at least, from haemoglobin, and are connected with its excretion through the kidney.

Attempts to demonstrate the existence of iron in the epithelial cells lining the tubules of these kidneys were unsuccessful. Here again, however, the explanation may be that the haemoglobin had not sufficiently broken down for liberation of the iron from its proteid combination to have occurred. It is suggestive in this connection to note that Stieda (1893) has described in the kidneys of blackwater fever patients the existence of granules in the epithelium of the convoluted tubes, and states that these granules gave the iron reaction.

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DESCRIPTION OF PLATE XVII

The tissues were fixed in formalin and the sections stained by Heidenhain's iron alum haematoxylin method.

Fig. 1.—Section of renal cortex of a pup which died from *Piroplasma canis* during the passage of haemoglobinuria. In the epithelium lining the convoluted tubules are numerous darkly stained granules varying considerably in size. The granules are not present in Bowman's capsule.

Fig. 2.—Section of renal cortex of a rabbit four hours after the intravenous injection of haemoglobin. This kidney presents the same appearances as are shown in Fig. 1.

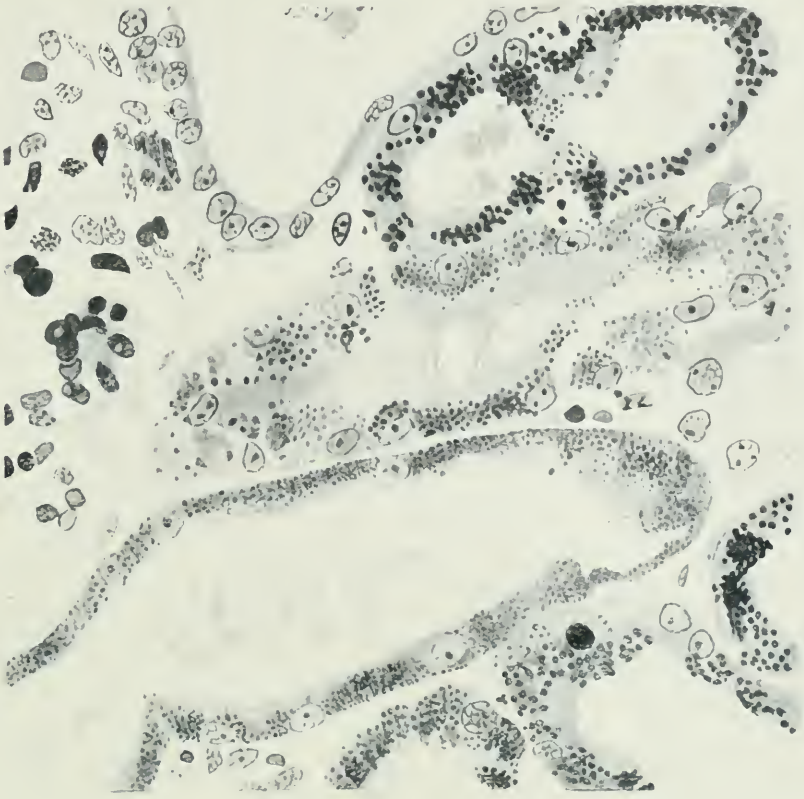


FIG. 1.

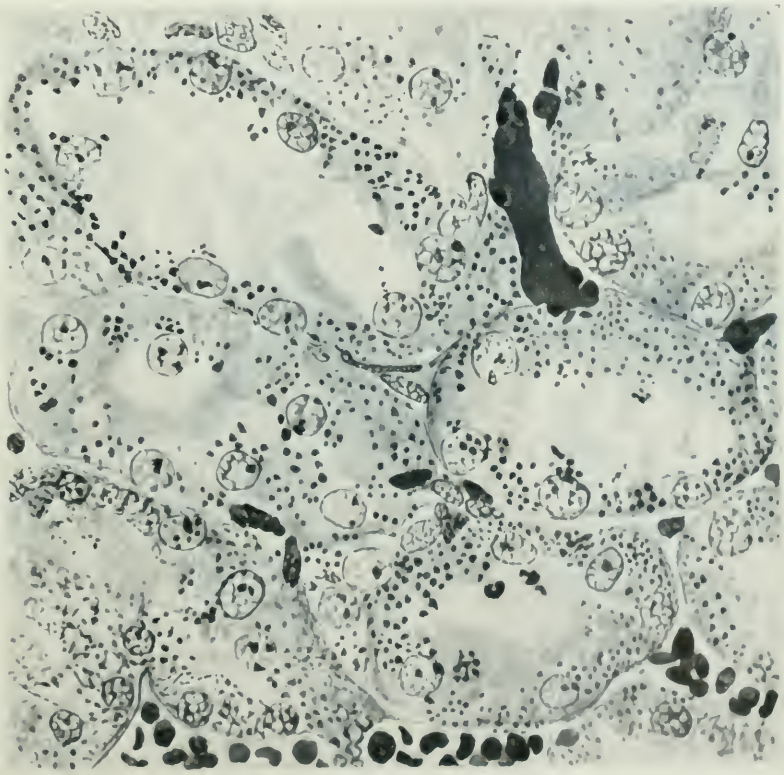


FIG. 2.

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